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Sharon L. Turner, Ph.D.
CM1-8A12 GAU 1647
(703) 308-0056

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Proteolytic Digestion Patterns of "Soluble" and "Detergent-Soluble" Bovine Caudate Nucleus Acetylcholinesterases

David J. Marsh and Jean Massoulié

Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France

Abstract: The structures of purified "soluble" and "detergent-soluble" bovine caudate nucleus acetylcholinesterases were compared by peptide mapping on polyacrylamide gels. The digestion products generated from the two acetylcholinesterases on proteolysis by a given protease (*Staphylococcus aureus* V8 protease, α-chymotrypsin, or papain) are remarkably similar as judged from the electrophoretic band patterns. We conclude that the "soluble" and "detergent-soluble" acetylcholinesterases from bovine caudate nucleus share a common evolutionary origin. **Key Words:** Acetylcholinesterase—Bovine caudate nucleus—Polymorphism—Peptide mapping—Sequence homology. **Marsh D. J. and Massoulié J.** Proteolytic digestion patterns of "soluble" and "detergent-soluble" bovine caudate nucleus acetylcholinesterases. *J. Neurochem.* **44,** 1602–1604 (1985).

The extensive polymorphism of mammalian acetylcholinesterase (AChE, EC 3.1.1.7) has been well documented (Massoulié et al., 1980, 1984; Massoulié and Bon, 1982). Mammalian brain acetylcholinesterase consists almost exclusively of globular forms, principally the tetramer (Massoulié et al., 1980, 1984; Massoulié and Bon, 1982; Grassi et al., 1982). Extraction of acetylcholinesterase from brain tissue is generally performed in two stages: First, the tissue is homogenized in the presence of detergent-free buffer. After centrifugation the supernatant is operationally defined as "soluble," or S_1, acetylcholinesterase and generally represents 10–20% of the total acetylcholinesterase activity of the tissue. Second, the pellets obtained after the first extraction are homogenized in a buffer that contains detergent (usually 0.5–1.0% Triton X-100) and the activity extracted (the remaining 80–90%) is defined as "detergent-soluble," or S_2, acetylcholinesterase. The S_1 and S_2 acetylcholinesterase pools contain monomers, dimers, and tetramers in different proportions, with the tetramer predominating in both.

Because of intense interest in the role of acetylcholinesterase polymorphism it is of value to establish whether these two operationally defined extracts derive from structurally distinct enzyme pools and are distinguishable by physicochemical or immunological criteria. To this end various studies have been published on human (Sørensen et al., 1982), rat (Zanetta et al., 1981; Rakonczay et al., 1981), and bovine (Grassi et al., 1982) brain acetylcholinesterases. The S_1 and S_2 pools appear to be distinguishable by certain physicochemical criteria (Rakonczay et al., 1981; Sørensen et al., 1982), and some authors have also claimed that they are immunologically distinct, thus implying that the two acetylcholinesterase pools are the products of different genes (Zanetta et al., 1981; Sørensen et al., 1982).

A recent study from this laboratory is in disagreement with these latter results, as no immunological distinction could be drawn between the S_1 and S_2 acetylcholinesterases (Marsh et al., 1984). This finding suggests that the two enzyme pools derive from the same gene(s) or from gene(s) of common ancestry but it cannot exclude the possibility of distinct genetic origins.

We attempted to resolve this problem by a study of the primary structures of the subunits of the S_1 and S_2 acetylcholinesterase pools using the method of peptide mapping on polyacrylamide gels of pro-

Received July 30, 1984; accepted November 2, 1984.
Address correspondence and reprint requests to Dr. D. Marsh at Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 Rue d'Ulm, 75230 Paris Cedex 05, France.

Abbreviation used: SDS, sodium dodecyl sulfate.

teins subjected to partial proteolysis in the presence of sodium dodecyl sulfate (SDS) (Cleveland et al., 1977). Since its introduction this technique has become a widely used method for sensitive structural studies of related proteins. The patterns of peptide fragments generated by proteolysis are reproducible and characteristic of the protein substrate and the protease. This method allows then a direct comparison of the sequence homologies of different proteins.

MATERIALS AND METHODS

"Soluble" (S_1) and "detergent-soluble" (S_2) acetylcholinesterases from bovine caudate nuclei were extracted and purified as previously described (Vallette et al., 1983; Marsh et al., 1984). The purity of the enzymes was confirmed by SDS-polyacrylamide gel electrophoresis using a discontinuous system (Laemmli, 1970), and the subunit molecular weights were found to be approximately 76,000 by either silver staining (Wray et al., 1981) or by fluorography of the enzyme covalently labelled by tritiated diisopropyl fluorophosphate.

The purified enzymes were labelled with [^{14}C]-formaldehyde (Jentoft and Dearborn, 1979), dialyzed against 5 m*M* Tris-HCl, and the radioactivity in a given aliquot was measured. Assuming that an acetylcholinesterase activity of 100 optical density units/min is equivalent to approximately 2.5 μg of enzyme [estimated using an acetylthiocholine mammalian enzyme turnover number of 1.3×10^7 mol/h/site (Vigny et al., 1978) and a subunit molecular weight of 76,000] the radioactivity incorporated/μg of enzyme was calculated and fell in the range 30,000–40,000 cpm/μg. The dialyzed, ^{14}C-labelled enzymes were lyophilized and resuspended in 0.125 *M* Tris-HCl pH 6.8, 1 m*M* EDTA, 0.5% SDS at concentrations of approximately 0.1 mg/ml.

The enzymes were proteolytically digested at 37°C for 20 min using the following protease concentrations: papain 40 ng/ml, *Staphylococcus aureus* V8 protease 150 μg/ml, or α-chymotrypsin 750 μg/ml. Digestions were terminated by the addition of β-mercaptoethanol to 5% followed by boiling for 3 min. An equal volume of 0.125 *M* Tris-HCl, pH 6.8, 40% glycerol, 1% SDS, 1% β-mercaptoethanol, 0.01% bromophenol blue was added to each sample before loading onto the gel.

The digestion products (~10,000–40,000 cpm/well) were electrophoresed on 15% polyacrylamide slab gels until the bromophenol blue marker had reached the bottom of the gel (~1,000 V-h). Gels were fixed in a mixture of 10% trichloroacetic acid, 30% methanol, and 10% acetic acid, treated with a fluorographic enhancer (EN^{3}HANCE, New England Nuclear), dried, and placed with Kodak X-Omat AR film at −70°C for 1–3 weeks before development of the film.

RESULTS AND DISCUSSION

Figure 1 shows the proteolytic digestion patterns obtained from "soluble" and "detergent-soluble" bovine caudate nucleus acetylcholinesterases on digestion by *Staphylococcus aureus* V8 protease, by α-chymotrypsin, or by papain. Visual inspection shows that the proteases generate distinct band patterns over the 76,000–10,000 molecular weight range contained in the gel. Consistent patterns were obtained with several enzyme preparations and a variety of protease concentrations. It should be noted that more than one subunit polypeptide chain of similar molecular weight may be present in the enzyme preparations. If this polypeptide microheterogeneity does exist the band patterns obtained will clearly derive from the digestion of a mixed polypeptide population.

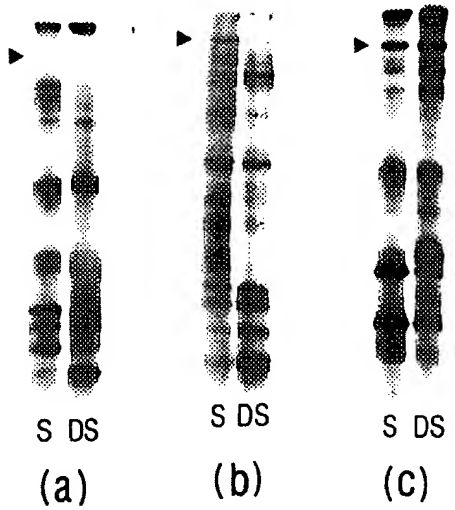

FIG. 1. Peptide maps of "soluble" and "detergent-soluble" bovine caudate nucleus acetylcholinesterases digested by **(a)** papain, **(b)** α-chymotrypsin, or **(c)** *S. aureus* V8 protease. The arrowheads indicate the position of the 76,000-dalton subunit. See Materials and Methods for digestion conditions.

The patterns obtained for the "soluble" and "detergent-soluble" acetylcholinesterases, whether digested by papain (Fig. 1a), α-chymotrypsin (Fig. 1b), or *S. aureus* V8 protease (Fig. 1c), are essentially indistinguishable. The peptide maps indicate, therefore, that the "soluble" and "detergent-soluble" enzymes share an extensive common amino acid sequence.

The considerable sequence homology demonstrated by the peptide maps presented here is indicative of a common genetic origin for the "soluble" and "detergent-soluble" acetylcholinesterases. This result is in agreement with our previous conclusions based on immunological data (Marsh et al., 1984) but in contrast with the interpretations presented by other authors on the basis of their immunological studies of rat (Zanetta et al., 1981; Rakonczay et al., 1981) and human (Sørensen et al., 1982) brain "soluble" and "detergent-soluble" acetylcholinesterases.

The apparent discrepancies in the various immunological studies were examined in detail in our previous report (Marsh et al., 1984). The data pre-

sented here are a direct reflection of acetylcholinesterase structures and are assumed to be free of the difficulties that may accompany the interpretation of immunological data. For this reason we conclude that the "soluble" and "detergent-soluble" acetylcholinesterases from bovine caudate nuclei share a common evolutionary origin. This finding may be applicable to mammalian brain "soluble" and "detergent-soluble" acetylcholinesterases in general.

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